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Chromosomes and their meiotic behaviour in two species of *Dieuches* Dohrn, 1860 (Heteroptera: Lygaeidae: Rhyparochromini)

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Abstract. The Lygaeidae (Heteroptera) are a large and diverse family in which the male diploid chromosomal complement ranges from 10 to 30. Diploid numbers of 14 and 16 are taken as two modal numbers of the family. The Rhyparochrominae, one of the largest subfamilies of the Lygaeidae, are known to be heterogeneous both cytologically and morphologically. Available data on the tribe Rhyparochromini reveal that all species are characterized by the presence of a pair of microchromosomes (m-chromosomes) and have an XY/XX (♂/♀) sex chromosome determining system. *Dieuches coloratus* (Distant, 1909) and *D. insignis* (Distant, 1918) belonging to Rhyparochromini, have 2n=14=10A+2m+XY and 2n=12=8A+2m+XY respectively. Both the species are similar in having one pair of distinctly large autosomes in their chromosome complements. The metaphase plate arrangement of autosomes, sex chromosomes and m-chromosomes in *D. coloratus* is similar to the common condition observed in the tribe Rhyparochromini. In *D. insignis*, however, the arrangement is different. Here, metaphase I is usual in showing peripheral position of autosomes and central position of sex chromosomes and m-chromosomes. At metaphase II, however, autosomes, sex chromosomes and m-chromosomes are peripherally placed, an arrangement, which is not reported earlier in the tribe Rhyparochromini.

Key words: Lygaeidae, Rhyparochromini, *Dieuches*, chromosomes, meiosis, metaphase plate arrangement.

Introduction

The Lygaeidae are a large and diverse family of the suborder Heteroptera. This taxon was first recognized as a higher group by Schilling (1829), and the first and most complete early synthesis was performed by Stål (1872). It was Slater (1964) who provided a modern world catalogue of the Lygaeidae. So far, 16 subfamilies comprising at least 500 genera and approximately 4000 valid species are distributed in all regions both in Old and New Worlds (Schuh, Slater, 1995). Most of the lygaeids, commonly named as seed bugs, are plant feeders and usually occur on low plants and underneath stones and fallen leaves.

Ueshima and Ashlock (1980) and then Grozeva and Kuznetsova (1993) provided comprehensive data on the cytotaxonomy of the subfamilies of the Lygaeidae. The subfamily Rhyparochrominae is one of the largest subfamilies and is known to be heterogeneous both cytologically and morphologically. In the tribe Rhyparochromini, 32 species have been cytologically investigated as per the data compiled by Grozeva and Kuznetsova (1993). Cytological data provided by Ueshima and

Ashlock (1980) reveal that all species are characterized by the presence of a pair of microchromosomes (m-chromosomes) and have an XY/XX (♂/♀) sex chromosome determining system. Fifteen species have the modal diploid number of 14, seven have 2n=12 and one has 2n=10. Three species of *Poeantius* Stål, 1865 studied so far, however, lack the Y chromosome; *Graptopeltus japonicus* Stål, 1874, *Megalonotus antennatus* Schilling, 1829 and *Sphragisticus nebulosus* Fallén, 1807 carry multiple X-chromosomes, while *Rhyparochromus angustatus* (Montandon, 1893) males have two Y chromosomes (Ueshima, Ashlock, 1980).

In India, significant cytological work has been done on the Lygaeidae up to the mid sixties (Manna, 1951, 1958, 1962; Parshad, 1957; Banerjee, 1958; Jande, 1959a, b) but afterwards only scattered works are available (Manna, Deb-Mallick, 1981; Barik et al., 1981; Dey, Wangdi, 1988; Satapathy, Patnaik, 1989). There is a wide gap in the cytological information about Heteropteran species from India. The present authors have taken up a project funded by Department of Science and Technology (DST), New Delhi to carry out cytological studies on Heteropteran fauna of India in an attempt to fill the gap and to add further cytological information to the knowledge existing so far. The present paper, a part of the project, pertains to chromosome complement and meiotic behaviour of two lygaeids, *Dieuches coloratus* (Distant, 1903) and *D. insignis* (Distant, 1903) (Rhyparochrominae: Rhyparochromini) collected from Punjab, India.

Material and Methods

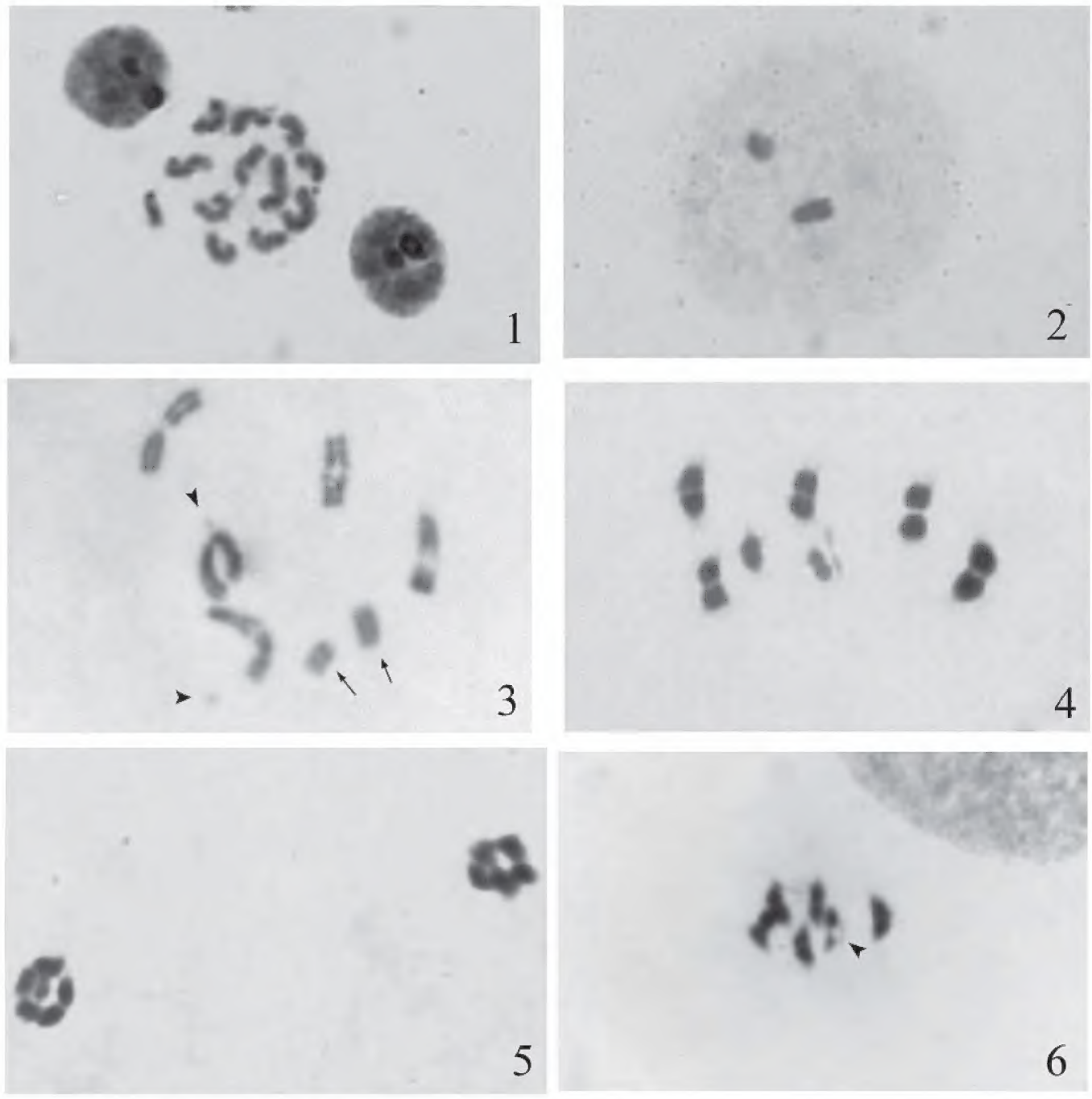
Male specimens of *Dieuches coloratus* were collected using a light trap in the campus area of Punjabi University, Patiala, Punjab, India while those of *D. insignis* were collected from fallen leaves and underneath wet logs in an abandoned forest in Ropar district of Punjab, India. Live specimens were dissected to take out testes which were fixed in Carnoy's mixture (absolute alcohol: acetic acid, 3:1) and given two successive changes, each of 15 minutes. Chromosome preparations were made using air-drying method and preserved in refrigerator. About four-week old slides were stained in carbol fuchsin and differentiated in n-butanol, dried at room temperature and finally mounted in DPX for microscopic examination.

Results

Dieuches coloratus

The chromosomal complement consists of 14 elements i.e. 2n=10A+2m+XY. One of the autosomal pairs is distinctly larger than the rest of the autosomes which are of almost equal size. The X is rod shaped and is nearly equal in size to the largest autosome while the Y is equal to the smaller autosomes. Microchromosomes are the smallest dot shaped elements (Fig. 1). At the diffuse stage which is characteristic of the suborder Heteroptera in general (Ueshima, Ashlock, 1980), two heteropycnotic bipartite bodies are recognized, one large corresponding to the X and the other small corresponding to the Y (Fig. 2). At diplotene, five autosomal bivalents, two well-separated faintly stained m-chromosomes and the X and Y univalents are observed. All the bivalents show a single terminal chiasma (Fig. 3).

Metaphase I plates show five autosomal bivalents arranged roughly in a circle while two bipartite sex chromosomes and the microchromosomal pair lie in the centre. The autosomes and the sex chromosomes appear isopycnotic while m-chromosomes are faintly stained and separate precociously (Fig. 4). At telophase I, five autosomes appear in a ring while the X and



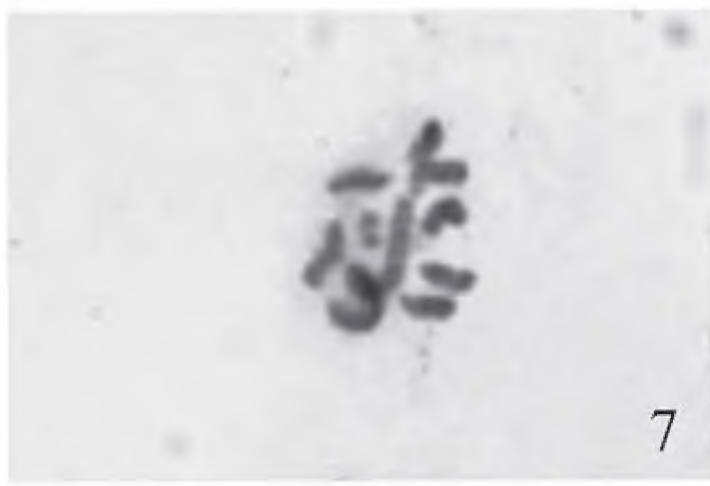
Figs 1-6. Chromosomal complement of *Dieuches coloratus*, 2n = 10A+2m+XY. **1**- spermatogonial prometaphase. **2** - diffuse stage. **3** - late diplotene. **4** - metaphase I. **5** - telophase I. **6** - metaphase II. Arrows show sex chromosomes and arrowheads show m-chromosomes. Bar = 10 µm.

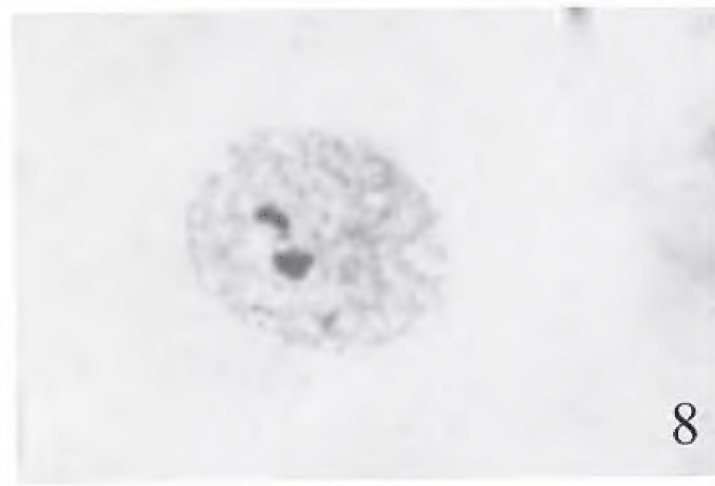
Y join to form a pseudobivalent that lies in the centre. The microchromosomes are not visible (Fig. 5). At metaphase II, five autosomes lie again in a circle while the head and tail shaped XY pseudobivalent along with a bipartite microchromosome lie in the centre (Fig. 6).

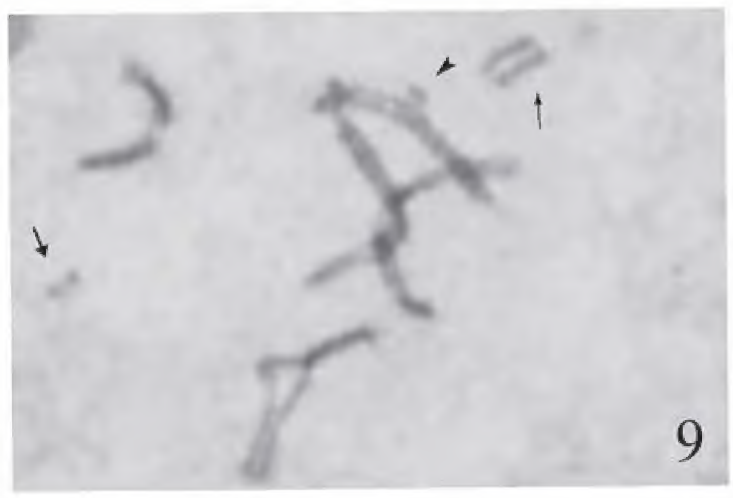
Dieuches insignis

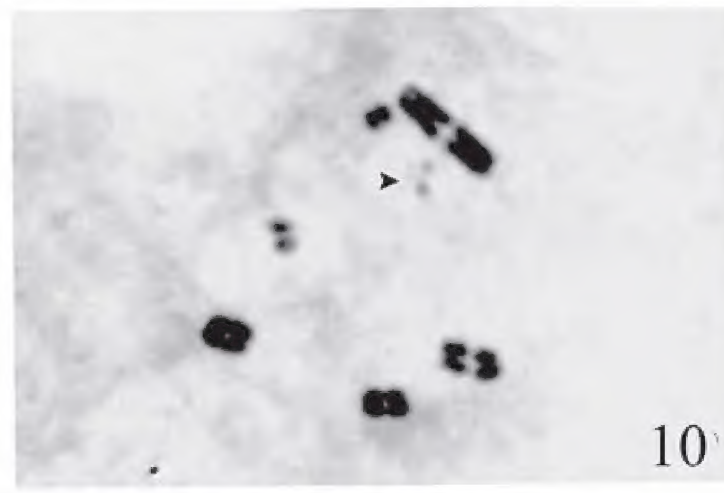
The chromosomal complement consists of 12 elements i.e. 2n=8A+2m+XY. On the basis of their size, chromosomes can be divided into four groups: the first group contains the largest autosomal pair, the second group has two pairs of smaller autosomes and the X chromosome; the third group has one smallest autosomal pair and the Y chromosome while the fourth group has one pair of dot shaped m-chromosomes (Fig. 7). During diffuse stage, X and Y appear bipartite and heteropycnotic (Fig. 8). Diplotene shows four autosomal bivalents, two faintly stained unpaired m-chromosomes and the bipartite X and Y chromosomes. Each bivalent has a single chiasma which can be terminal, subterminal or interstitial (Fig. 9). At diakinesis, the condensed bivalents show a terminal chiasma and the m-chromosomes come close to form a pseudobivalent (Fig. 10).

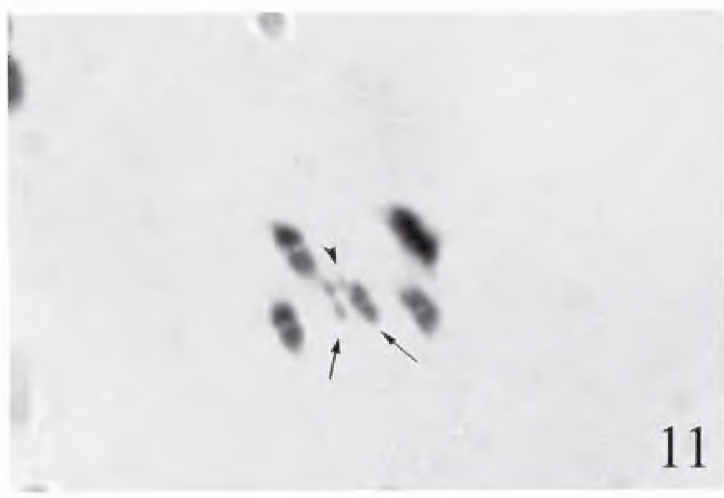
At metaphase I, bivalents lie roughly in a

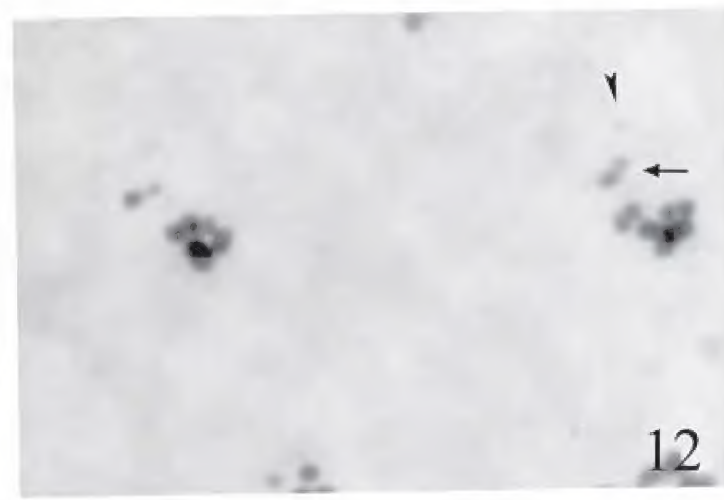


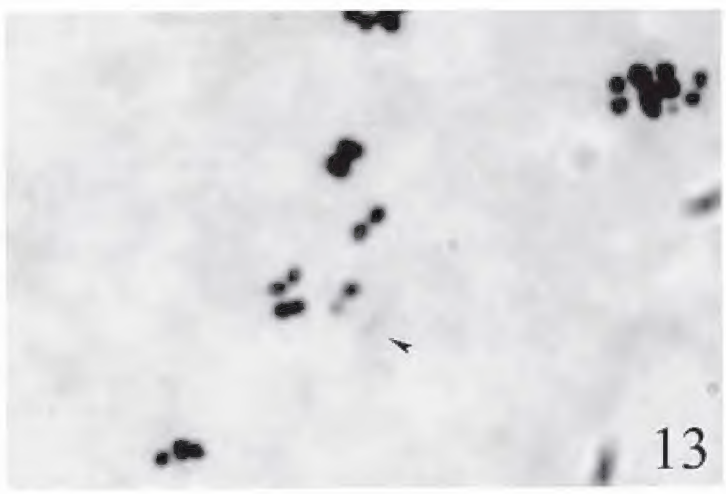


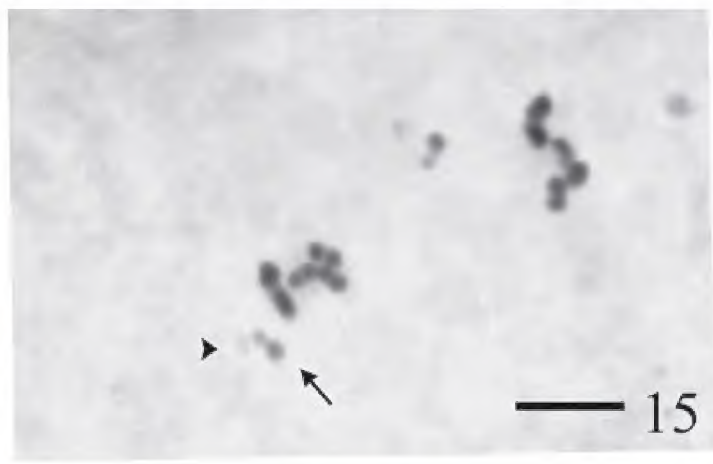












Figs 7-15. Chromosomal complement of *Dieuches insignis*, 2n=8A+2m+XY. **7** - spermatogonial prometaphase. **8** - diffuse stage. **9** – diplotene. **10** – diakinesis. **11** - metaphase I. **12** - telophase I. **13** - metaphase II (polar view). **14**, **15** - metaphase II (side view). Arrows show sex chromosomes and arrowheads show m-chromosomes. Bar = 10 μm.

circle while sex chromosomes and m-chromosomes lie in the centre of the ring (Fig. 11). At telophase I, the sex chromosomes form a pseudobivalent which generally lies outside the autosomal group. Microchromosomes mostly remain close to the XY pseudobivalent (Fig. 12). At metaphase II, polar views show peripheral position of pseudobivalent and m-chromosomes along with autosomes (Fig. 13). Side viewed plates clearly show the outer position of the pseudobivalent and the m-chromosomes placed generally at a distance from the autosomes (Fig. 14, 15).

Discussion

The male diploid chromosome complements in the Lygaeidae known so far range from 10 to 30. Diploid numbers of 14 and 16 are taken as two modal numbers in the family. In the subfamily Rhyparochrominae, six types of chromosome complement are reported, namely 2n=12, 14, 16, 18, 20 and 22 (Ueshima, Ashlock, 1980; Grozeva, Kuznetsova, 1993). The tribe Rhyparochromini is cytologically the most heterogeneous group with 14, 16 and 18 as equally frequent diploid numbers, while in the tribe Beosini 2n=12, 14 and 16 are found and 2n= 20 is restricted to the tribe Lethiini (Manna, 1951). On the other hand, Ueshima and Ashlock (1980) found 14 to be the most frequent, 12 to be fairly reported and 10 to be the least represented number in the tribe Rhyparochromini. Microchromosomes are invariably present. In the entire tribe, XY is the most common sex determining mechanism. Three species of *Poeantius* studied so far, however, lack the Y chromosome; *Graptopeltus japonicus*, *Megalonotus antennatus*, and *Sphragisticus nebulosus* carry multiple X-chromosomes, while *Rhyparochromus angustatus* males have two Y chromosomes (Ueshima, Ashlock, 1980). Multiple sex chromosomes are also present in the tribes Beosini and Lethiini (Pfaler-Collander, 1941).

D. coloratus and *D. insignis* from the present study have 2n=14=10A+2m+XY and 2n=12=8A+2m+XY, respectively. Both of the species are similar in having a pair of m-chromosomes and XY as the sex determining mechanism, which are the two main characteristics of the tribe Rhyparochromini. However, they differ in the number and size of autosomes. Their chromosome complements show similarity in having one distinctly large autosomal pair. The rest of the chromosomes are almost of equal size in *D. coloratus* but show further gradation in *D. insignis*. Chromosomes of *D. coloratus* are comparatively larger than the chromosomes of *D. insignis*. In species of *Dieuches* studied so far, 12 is the most frequent number being reported in *D. unigguttatus* (Thunberg, 1822) by Manna (1951) and in other unidentified species by Parshad (1957), Ueshima and Ashlock (1980), Manna and Deb-Mallick (1981), Dey and Wangdi (1988) and Satapathy and Patnaik (1989). Malipatil (1979) reported 14 and 16 chromosomes in *D. notatus* (Dallas, 1852) and *D. maculicollis* (Walker, 1872), respectively, while Ueshima and Ashlock (1980) reported 14 in *D. basiceps* Eyles, 1973

An exceptionally large autosomal pair is commonly reported in the family Lygaeidae (Ueshima, Ashlock, 1980). In Orsillinae and Blissinae, the extremely large chromosome is found in all the species with 2n=14 but species with 2n=16 lack this exceptionally large chromosome in the complement, which indicates that change from 14 to 16 in these two subfamilies took place by fragmentation of the largest chromosome. An exception to this condition is found in *Ischnodemus notandus* Slater and Wilcox, 1969 (Blissinae) with 2n= 18 and *Ortholomus arphnoides* Baker, 1906 (Orsillinae) with 2n= 16, both of which retain the distinctly large autosome indicating that

increased chromosomal number is a result of fragmentation of any other autosome rather than that of the largest one. The species with 12 chromosomes are thought to have originated as a result of fusion of two autosomes (Ueshima, Ashlock, 1980). An interesting evolutionary behaviour is observed in *Dieuches* (Rhyparochromini). Within this genus, the number of 12 seems to be the basic one as it is the most frequent number followed by 14 and then 16 (Table). One pair of exceptionally large chromosomes is present invariably in all the species of *Dieuches* having 2n=12 (Manna, 1951; Parshad, 1957; Ueshima, Ashlock, 1980). Out of two species with 2n=14, it is retained in *D. notatus* (Malipatil, 1979) but is absent in *D. basiceps* (Ueshima, Ashlock, 1980) while in *D. maculicollis* with 2n=16, it is absent (Malipatil, 1979). The species with higher chromosomal number are thought to have originated as a result of fragmentation of the largest chromosomal pair. However, in *D. coloratus* with 2n=14 and *D. insignis* with 2n=12, fragmentation or fusion seem to be unlikely as both the species retain the largest autosomal pair which is almost of equal size in both of them. The comparison of their complements does not indicate interchange in number due to fragmentation or fusion of any other chromosome too as the size of the rest

Table. Chromosomal complements of various species of *Dieuches* studied so far.

Species	2n	Exceptionally large chromosome pair	Reference
Dieuches uniguttatus (Thunberg, 1822)	12	Present	Manna, 1951
Dieuches sp.	12	Present	Parshad, 1957
Dieuches sp.	12	Not mentioned	Dey, Wangdi, 1979
Dieuches sp.	12	Not mentioned	Manna, Deb-Mallick, 1981
Dieuches maculicollis (Walker, 1872)	16	Absent	Malipatil, 1979
Dieuches notatus (Dallas, 1852)	14	Present	Malipatil, 1979
Dieuches sp. (PDA-14)	12	Present	Ueshima, Ashlock, 1980
Dieuches sp., probably *D. scioensis* Lethierry	12	Present	Ueshima, Ashlock, 1980
Dieuches basiceps Eyles, 1973	14	Absent	Ueshima, Ashlock, 1980
Dieuches sp.	12	Not mentioned	Satapathy, Patnaik, 1989
Dieuches coloratus (Distant, 1909)	14	Present	Present study
Dieuches insignis (Distant, 1918)	12	Present	Present study

of the chromosomes is larger in *D. coloratus* (2n=14) than *D. insignis* (2n=12).

In both species, meiosis is post-reductional with respect to sex chromosomes, which divide equationally during anaphase I and reductionally during anaphase II. Chromosomal behaviour during meiosis in Rhyparochrominae is rather heterogeneous. In one condition, autosomes form a ring while the sex and the m-chromosomes lie in the centre both during metaphase I and metaphase II. This is prevalent in tribes Lethaeini (except *Lethaeus* Dallas, 1852), Ozophorini, Antillocorini (except *Cligenes* Distant, 1893), Drymini, Stygnocorini and Rhyparochromini (except *Dieuches uniguttatus* (Thunberg, 1822), *Peritrechus* Fieber, 1861 and *Poeantius*). In the second prevalent condition, autosomes lie in a ring while the sex and micro-chromosomes are placed centrally at metaphase I but at metaphase II, m-chromosomes are peripheral with autosomes while the sex chromosomes occupy the centre. This condition is found in the tribes Plinthisini, Cleridini, Gonianotini, Myodochini and in *Dieuches uniguttatus*, *Peritrechus*, and *Poeantius* of Rhyparochromini (Manna, 1951; Ueshima, Ashlock, 1980). The position of autosomes, sex chromosomes and m-chromosomes at metaphase I and metaphase II in *D. coloratus* is similar to the first condition, which is commonly observed in the tribe Rhyparochromini. In *D. insignis*, however, the arrangement is different. It does not match even with the second prevalent condition found in exceptional cases of *D. uniguttatus*, *Peritrechus*, and *Poeantius* of Rhyparochromini. Here, metaphase I is usual in showing a central position of the sex chromosomes and m-chromosomes but during telophase I, the sex and m-chromosomes come to lie outside the autosomal ring. At metaphase II, autosomes, XY pseudobivalent and m-chromosomes all are peripherally placed but the m-chromosomes and sex chromosomes lie at a distance from the autosomes as is evident in side view of some metaphase II plates. This is a unique departure from the general condition, not even matching with earlier departures reported in Lethaeini (X peripheral at metaphase I) and Antillocorini (m peripheral at metaphase I and central at metaphase II) by Ueshima and Ashlock (1980). To confirm the observations, slides were prepared from nine specimens of *Dieuches insignis*. Similar behaviour was recorded in each specimen, which indicates that this unique behaviour is not merely an incidental departure but is well established in nature for this species.

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